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Diagnosis of *Mycobacterium tuberculosis*

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1. Introduction

Tuberculosis (TB), caused by the intracellular bacterium, *Mycobacterium tuberculosis* (Mtb), has been a major health concern since it plagued ancient Egypt 5 thousand years ago. TB infects 9 million people every year, most of them children (especially in endemic areas), and it leads to approximately 2 million deaths annually (World Health Organization [WHO], 2008; Kabra & Lodha, 2004; Marais & Pai, 2007). These numbers are expected to increase in the coming years because of (1) the AIDS pandemic—a high percentage of the patients with human immunodeficiency virus (HIV) are co-infected with Mtb, and (2) the emergence of drug-resistant strains of the TB organisms (Corbett et al., 2003; Raviglione, 2003; WHO, 1994). This alarming increase in morbidity and mortality highlights the need to strengthen control measures. Accurate and rapid diagnosis is essential for controlling the disease, yet the traditional tests for TB produce results that are either inaccurate or take too long to be definitive. A fast and reliable diagnostic method that could differentiate between active and latent TB infection is lacking as well.

The current routine diagnostic tests for TB: sputum smear microscopy, chest X-ray, Mtb culture, tuberculin skin test, acid-fast staining, and serological tests—all have their limitations. Sputum smear microscopy can produce false negative results, whereas the acid-fast staining requires a large number of bacteria in the sputum to give an accurate reading; a chest X-ray alone is inconclusive; Mtb culture takes too long to produce a result; the tuberculin skin test lacks specificity and reliability; and serological tests, which use different TB antigens to detect Mtb infection, are fast but they lack the necessary sensitivity.

The only available TB vaccine is the bacille Calmette Géurin (BCG) vaccine, which is uneven in its efficacy. Various reports have indicated variable levels of protection ranging from 0 percent to 80 percent in different populations (Fine, 1995; Tuberculosis Research Centre [ICMR], 1999). Therefore, despite the fact that most people in developing countries are vaccinated with BCG at birth, TB is still a major public health problem. The prevalence of TB infection is reported as being as high as 40 percent worldwide, and the annual risk of infection is 2–4 percent worldwide (Anil, 1995).

Clinically, TB has two forms: An active form and a latent form (which is asymptomatic and non-contagious). If undiagnosed and untreated, a patient with active pulmonary TB will transmit the infection to 10–15 people each year (WHO, 2006). However, active TB is also fueled by the vast reservoir of latent TB infections that become reactivated. Immunocompetent individuals latently infected with Mtb have a 10 percent lifetime risk of

developing active TB (Syblo, 1980; Harada, 2006). Data show that 5 percent of latently infected individuals will progress to active TB in the first 2 years after acquiring the infection, and an additional 5–10 percent of infected people will develop the active disease later in their lives (Comstock et al., 1974). This risk increases for people co-infected with HIV—especially children, for whom diagnosis of TB is even more challenging (Corbett et al., 2004).

If diagnosed, latent TB infected individuals can be cured with anti-tuberculosis treatment, which prevents progression to the active form of the disease. Because effective TB control can only be achieved with the accurate diagnosis and treatment of both active and latent infections, modern TB control programs require the identification of latent TB infection to the highest clinical standards. Accurate diagnosis and preventive treatment of latently infected individuals can substantially decrease the chance of development into active TB (Cohn, 2000). Delayed diagnosis, because of inaccuracy or the unavailability of diagnostic requirements—including the availability of rapid and accurate diagnostic methods—can preclude timely therapy, which may result in increasing morbidity and mortality, greater lung damage resulting in chronic disability, and higher health care costs (Kehinde et al., 2005; WHO, 2009).

The diagnosis dilemma for clinical TB continues to be a global issue. For pulmonary TB, it can be difficult to obtain robust respiratory specimens from the elderly, the young, and immuno-compromised patients. For those with extra-pulmonary TB, tissue biopsy is essential for histopathological and microbiological diagnosis (Bukhary & Alrajhi, 2004), yet techniques to obtain and examine biopsies are not available in all hospitals and may be associated with complications. A simple, noninvasive, rapid, and accurate method of diagnosis needs to be developed for successful treatment of both active and latent TB; with such a method, person-to person transmission of the disease would be greatly reduced, which would have a major impact on TB morbidity and mortality worldwide (Cambanis et al., 2007).

1.1 Specimen collection

For the detection of pulmonary and/or extrapulmonary TB, tests usually require sputum, gastric lavage, blood, urine, or other bodily fluids (such as cerebrospinal fluid, pleural, or ascetic fluid); in addition, tissue biopsy specimens are collected for the diagnosis of extrapulmonary TB. One of the objectives of developing TB biochemical markers and immunological assays is to replace the need for collecting tissue biopsy specimens from TB patients for diagnosis of the disease. Likewise, the development of immunochromatography tests (ICT), for which urine is used as a specimen, is an attempt to make diagnostic tests less invasive and costly, more rapid, and patient friendly. Up until now, regardless of which test is used, that test is usually accompanied by microbiological tests (smear microscopy and mycobacterial culture of sputum) to diagnose pulmonary TB and to determine the treatment be used. Sputum samples, after being collected, are decontaminated by using normal sodium hydroxide-N-acetyl-L-cystein (NaOH-NALC) and then sometimes centrifuged to get a better yield of the organisms (Kent & Kubica, 1985). Often three sputum samples are collected, including a "spot" specimen collected on the first day (first sputum); a morning (second sputum); and "spot" specimen (third sputum) collected on the second day. However, collecting adequate amount of sputum from patients is not always possible,

especially in children younger than 10 years old or in adults who cannot produce enough sputum. In situations like these, procedures to stimulate cough with an aerosol solution and/or bronchoalveolar or gastric lavage can be used (Capelozzi et al., 2011; Mohan et al., 1995; Somu et al.,1995).

2. Traditional TB diagnostic tests and their associated problems

Traditional TB diagnosis usually requires high clinical presentation, laboratory materials, and methods for sputum smear microscopy (acid-fast bacilli), culture on solid and/or liquid media, chest radiography, and the tuberculin skin test. Tissue sampling is usually needed to confirm preliminary results in cases of extra-pulmonary TB. All of these tests have their respective shortcomings.

2.1 Sputum smear microscopy

Sputum-smear microscopy is 100 years old, but it is still the primary, easy to use, and affordable test for the confirmation of pulmonary TB at the lower level of health services. Acid-fast bacilli smear Ziehl-Neelsen (ZN) microscopy, which is prepared from unconcentrated sputum (direct smear), is the main laboratory tool supporting case detection. It is inexpensive and is relatively specific in settings where tuberculosis is endemic. However, direct smear microscopy can produce false-negative results, which have been observed in more than 30–50 percent of adult patients (Miorner et al., 1994; Daniel 1990), particularly in high HIV-prevalent settings (Elliot et al., 1993; Frieden et al., 2003; American Thoracic Society Workshop, 1997), and 85–90 percent of infected children (Newton et al., 2008). The acid-fast bacilli false-negative result rate is attributable, in part, to the low sensitivity of the test, which requires more than 10,000 bacilli per milliliter of sputum for reliable detection (Perkins, 2000). Obtaining good sputum samples can be difficult, and the studious attention of trained and motivated technicians (who are not always available) is necessary.

Many attempts have been made to improve and optimize the performance of smear microscopy, including with new technologies (Mase et al., 2007; Bonnet et al., 2007; Ramsay et al., 2009; Mabaera et al., 2007; Van Deun et al., 2004), such as fluorescence microscopy, which uses inexpensive light-emitting diodes (LED) as an alternative for conventional ZN microscopy. This substitution increases the sensitivity of the test and is easy to use, even in peripheral laboratories where culture facilities are not available (Hooja et al., 2011; Steingart et al., 2006a; Steingart et al., 2006b; Steingart et al., 2007; Van Deun et al., 2008; Trusov et al., 2009; Minion et al., 2009; Bonnet et al., 2011). In fact, the World Health Organization (WHO) Strategic and Technical Advisory Group (STAG) for TB recommended that fluorescence microscopy be phased in as an alternative for ZN (WHO, 2009), because it can be used even in low-income, high TB burden settings. It has been reported that LED fluorescence microscopy, either alone or in combination with single-specimen tests, could increase considerably the identification of smear-positive cases (Cattamanchi et al., 2010).

A wide variety of stains or fluorescence quenchers have been used with the LED fluorescence microscopy; however potassium permanganate at 0.5 percent in water is the stain most frequently used. Although potassium permanganate can produce very good results with the classical fluorescence microscopy systems (using mercury vapor lamps and

epifluorecence), the very dark background sometimes makes it difficult to focus. Methylene blue (Mblue) is an alternative to potassium permanganate, which yields comparable results (Van Deun et al., 2010).

In an attempt to improve the performance of smear microscopy, sputum processing methods using household bleach (NaOCl), followed by a specimen concentration step (such as centrifuge or sedimentation, mentioned above), can be done in any laboratory setting before smear microscopy is used (Steingart et al., 2006; Angeby et al., 2004; Annam et al., 2009). However, some reports indicate that NaOCl sedimentation did not improve the performance of LED fluorescence microscopy in the diagnosis of pulmonary TB at low levels of health service in resource-poor countries (Bonnet et al., 2011).

Because the acid-fast bacilli smear is based on sputum, extra-pulmonary TB detection varies with the cytomorphology of inflammation at the site of infection, which is limited and may not exceed 40 percent (Nigussie et al., 2010; Gangane et al., 2008). The sputum smear microscopy test may also identify certain types of bacteria that are not Mtb, thus yielding a false-positive result for TB. The WHO estimates sputum smear microscopy only identifies 35 percent of patients with TB (Harris, 2004; Thornton et al., 1998). Furthermore, the 2010 WHO report indicated that in 2009, 43 percent of the 4.6 million reported new cases of pulmonary TB were diagnosed without microbiological confirmation (WHO, 2010). The failure to confirm TB infection can delay initiation of the appropriate therapy to adequately treat cases, which could prevent the further spreading of the disease (Cambanis et al., 2007).

2.2 Solid or liquid cultures

Solid or liquid cultures are still seen as the gold standard for TB detection because they are sensitive to live Mtb in the sputum sample; they can also provide data on the likely effectiveness of certain chemotherapeutic agents against TB. However, there are serious drawbacks to this test, such as the time needed to obtain the result (3–8 weeks). Clinical and therapeutic decisions are often made before the culture results are available. However, few facilities in low-income settings use culture for the diagnosis of TB; for those facilities the main method of diagnosis is sputum microscopy with acid. When culture *is* used in these settings, the Löwenstein-Jensen solid, egg-based, and agar-based Middlebrook 7H10 media are the ones used to recover mycobacteria from clinical materials (Metchock et al., 1999; Murray et al., 1998); these can take weeks to show results.

2.2.1 Advantages and disadvantages of cultures

A number of manual and automated systems have been developed to reduce the detection time of mycobacteria in clinical specimens. Both the biphasic Septi-chek acid-fast bacilli (Becton Dickinson, Sparks, MD) and the MB-Redox (BiotestAG, Dreieich, Germany) are examples of the manual systems. Advances in technology have led to the development of the automated systems such as radiometric BACTEC 460TB (Becton Dickinson), the fluorometric BACTEC MB9000 and BACTECMGIT (Mycobacteria Growth Indicator Tube), 960 systems (Becton Dickinson), the carbon dioxide–sensing MB/BacT ALERT 3D System (Organon Teknika, Durham, NC), and the pressure-sensing ESP Culture System II (Trek Diagnostic Systems, Westlake, OH). Detection time and isolation of Mtb were

considerably improved (7–21 days) with the use of liquid media, such as the radiometric BACTEC 460 TB broth-based system. However, this procedure still requires trained technicians and special attention to safety issues regarding radioisotopes (Salfinger & Pfyffer, 1994; Laszlo et al., 1983). Another disadvantage of the BACTEC 460 TB system is the increased cost of radioactive waste disposal, an issue that encouraged manufacturers to develop a better alternative. The fully automated BACTEC Mycobacteril Growth Indicator Tube (MGIT) liquid medium system with early growth indicators (the BACTEC MGIT 960 system), is faster and more sensitive than both LJ and BACTEC 460 TB for testing the susceptibility of antituberculosis agents, and it is more effective in diagnosing the disease in smear-negative samples; this feature shows great potential to reduce the mortality rate from TB (Lu et al., 2002; Gérôme et al., 2009; Sinirtas et al., 2009; Morcillo et al., 2010).

The BACTEC MGIT 960 system is a high-capacity, fully automated continuous-monitoring system, which can test up to 960 samples for the rapid detection of mycobacteria, making it suitable for those laboratories dealing with a large number of specimens (Somoskovi et al., 2000; Hanna et al., 1999; Tortoli et al., 1999; Lee et al., 2003). In the determination of the early bactericidal activity in the clinical studies of new anti-tuberculosis agents, it has been found that the time of detection of MGIT 960 is better than colony-forming units of Mtb on solid media (Diacon et al., 2010).

Although the WHO recently recommended the expanded use of liquid culture systems, such as MGIT, in resource-constrained settings (WHO, 2007), historically these systems have not been used because of the high cost of the tests and the culture contamination rates (Chihota et al., 2010). The relatively high contamination rates of the MGIT culture has been reported to range from 5.5 to 15 percent in high-income settings, and as high as 29.3 to 33 percent in resource-constrained settings (Chien et al., 2000; Lee et al., 2003; Hanna et al., 1999; Somoskvi et al., 2000; Chihota et al., 2010).

2.2.2 New approaches to cultures

Using simple and inexpensive monoclonal assays, such as the Capilia TB assay (a rapid and low-technology method), which uses monoclonal antibodies to detect a secreted mycobacterial protein (MPB64) during culturing (solid or liquid culture) allows it to differentiate Mtb from non-TB mycobacteria (Muyoyeta et al., 2010; Ngamlert et al., 2009). To shorten the time required for bacterial growth detection, Mtb can be isolated in both liquid- and solid-media cultures (Lu et al., 2002).

Although automated systems such as BACTEC 460 TB, BACTEC 9000, and MGIT can be used to accelerate the growth of the bacteria, they can also produce inaccurate results (Daniel, 1987). Plus, it is not always possible to obtain bacteria in the sputum sample. False-positives, which range from 0.1 percent to 65 percent because of laboratory contamination is another concern with the culture technique (Ruddy et al., 2002). Viable organisms can present additional problems, especially in patients who have started treatment. Therefore, even though culture has thus far been considered the gold standard for TB diagnosis, it still lacks the desired accuracy; it has been estimated that no more than 81 percent of the confirmed TB cases can be detected by culture (API, 2006).

2.3 Tuberculin Skin Test (TST)

Tuberculin skin testing (TST), also known as the Mantoux test or Heaf test, remains in widespread use for both the diagnosis of active TB and the detection of latent TB, and for the identification of TB in health care workers, for whom the incidence of TB is higher than in the general population (Harries et al., 1997; Barrett et al., 1979) and who require routine checkups for accidental acquisition of TB infection and chemoprophylaxis. The TST is a delayed type hypersensitivity skin test: an induration develops and is measured 48 to 72 hours after the intradermal inoculation of purified protein derivatives. It is generally accepted that in adults a TST response greater than or equal to a 10-millimeter induration is indicative of TB infection; however in children, the gauge differs in different settings. Importantly, the TST is still used as an epidemiological tool to screen for TB and to calculate the annual risk of TB through data generated by TST surveys. TST surveys are useful for detection of TB in communities with low case-detection rates, to assess the effect of HIV infection on a TB epidemic, and to better understand the effect of both diseases on children (Farhat et al., 2006).

2.3.1 Limitations of the tuberculin skin test

Although the TST is inexpensive, easily available, and is the preferred test in most TB-prevalent settings, it has a number of limitations. The TST is not patient friendly, in that it requires two visits to the health facility: the first visit is when the test is administered; and the second visit, 2 – 3 days later, is to assess the skin's reaction. It is estimated that one third of the people tested never return after the 48- to 72-hour waiting period to have their tests read (ATS, 2000; Lee & Holzman, 2002).

2.3.2 False positives

Purified protein derivatives contain more than 200 antigens shared with the BCG vaccine and many of the non-TB environmental mycobacteria, which can result in low specificity of the TST (Huebner et al., 1993; Dacso 1990; Diel et al., 2009; Pai et al., 2008). This cross-reactivity results in false-positive reporting for a large percentage of the world's population. Some reports indicate that BCG vaccination can present TST false-positive results for up to 15 years after vaccination (Wang et al., 2002). These variables contribute to the false positive results: (1) the strain and dose of BCG inoculated (Wang et al., 2002; Davids et al., 2006); (2) the method of vaccine administration (Davids et al., 2006); (3) the time since vaccination (Menzies, 2000); (4) the number of BCG scars (Babayigit et al., 2011); and (5) the weight and age at the time of vaccination (Newort et al., 2004). If the BCG vaccine was received in infancy, the impact on TST results is minimal, especially 10 or more years after vaccination. A person's nutrition at the time of vaccination as well as genetic factors can also have an impact on the outcome of the TST results later on (Newport et al., 2004). More frequent, more persistent, and larger TST reactions were observed in individuals who had received the BCG vaccine later in life, ie, after infancy (Pérez-Then et al., 2007; Farhat et al., 2006). Sometimes TST indurations between 5–10 millimeters can still develop for up to 25 years after vaccination (Miret-Cuadras et al., 1996). The TST false positive reaction was not associated with a family history of tuberculosis, with exposure to cigarette smoke, number of household family members, and the presence of respiratory allergic diseases (Babayigit et

al., 2011). A number of additional factors can contribute to false-positive results including inaccuracy of reading and documenting the results (Mancuso et al., 2008).

Furthermore, the TST does not distinguish between individuals infected with Mtb, vaccinated with BCG, or infected with environmental non-TB mycobacteria— almost one third of the people who test positive on the TST do not have a TB infection (American Thoracic Society [ATS], 2000; Huebner et al.,1993; von Reyn et al., 2001). Clinically non-TB mycobacteria rarely causes TST false-positives in low-prevalence settings of TB infection, however it does have an effect on the false-positive results of populations with a high prevalence of non-TB mycobacteria (Farhat et al., 2006). This lack of specificity (high rate of false-positive) in diagnosing both active and latent TB (WHO, 1995) is considered the TST's major drawback.

2.3.3 False negatives

The TST can also produce false-negative readings, and these can be product-related (associated with improper storage or handling). The number of tuberculin units inoculated and the type of tuberculin can have an effect on TST reactivity (Farhat et al., 2006). The sensitivity of the test is affected by the immunomodulation of the skin; the DTH response is influenced by illness or immunosuppression, and factors such as HIV infection or a young child's age can result in even lower sensitivity of the test for both latent and active TB (Swaminathan et al., 2008; Selwyn et al., 1992; Pesanti, 1994; Madariaga et al., 2007; Moreno et al., 2001).

2.3.4 The boosting effect

Other disadvantages associated with the TST include the "boosting effect," a phenomenon in which multiple TST administrations over time yield a false positive. The increased tuberculin reaction is seen in some individuals when a second skin test is administered 1 week to 1 year after administration of a first skin test that is nonreactive. This could be explained as an anamnestic recall of immune response that occurs in individuals with remote exposure to mycobacterial antigens. This phenomenon is a problem for people who are regularly screened for TB infection using the TST (for example, health care workers, hemodialysis patients, etc.) and become immunized to purified protein derivatives by the repeated administrations of the test (Dogan et al., 2005; Cengiz & Seker, 2006). Persistent negative TST in latent TB-infected individuals, despite the continued exposure, has been reported. It has been shown that this reaction can be attributed to genetic factors. These genetic factors not only influence the interaction between humans and Mtb but they can affect the outcome of the exposure: exposure but no infection, infection without progression, or progression to disease (Stein et al., 2008). Subjectivity and inter-individual variability, in the administration and reading of the TST can be added to the disadvantages and resultant errors, because it is difficult to administer small amounts of the protein uniformly; that is, the amount of purified protein derivatives delivered in the TST may vary, and this affects the size of the reaction (Chaparas et al., 1985).

Further research is needed to determine the best cut-offs for TST sensitivity, the optimal time for testing candidates, especially for people that need to be tested periodically (such as health care workers), and the cost-effectiveness of the test, given its limitations (Khawcharoenporn et al., 2011).

2.4 Chest X-ray

Chest radiography can be a useful tool to confirm TB when combined with a patient's history, physical exam, and laboratory tests in symptomatic and even smear-negative patients. Pulmonary TB almost always shows abnormalities on the chest radiograph; the pulmonary cavities and lesions are smaller when infected with TB than those caused by other chest health problems.

2.4.1 Disadvantages of chest X-ray

A chest X-ray cannot alone confirm a TB diagnosis. In many cases (40 percent), the infection is not in the lungs; radiography may not detect the early stages of TB disease, because the damage to the lungs may not yet be sufficiently marked to be detectable by a chest X-ray. Also, scarring in the lungs may be detected if previous TB disease has occurred (even if the patient is completely cured), and thus it is difficult to distinguish past cured TB from current TB disease.

2.4.2 Computerized Tomography (CT)

When both chest X-ray and computerized tomography were used to screen for latent TB in pre-transplant patients, abnormal findings were only detected on the chest CT (the chest X-ray results were normal), which indicates that chest CTs can detect latent TB better than chest X-rays (Lyu et al., 2011). Many studies have confirmed that CT has detected pulmonary TB cases that were missed by chest radiographs. Furthermore, high resolution CT alone, or CT together with the TST and INF-γ release assays, were effective in the differentiation between active TB and latent TB (Lee et al., 2010; Boloursaz, 2010). Even in sputum smear–negative sittings, high-resolution CT findings, such as tree-in bud appearance, lobular consolidation, and large nodules, accurately predicted the risk for pulmonary TB with reproducible results (Nakanishi et al., 2009).

2.5 Nucleic acid amplification test

The nucleic acid amplification test detects the nucleic acid specific to Mtb using an amplification technique (Noordhoek et al., 1995; Kadival et al., 1995; Nagi et al., 2007). Nucleic acid amplification is a relatively new assay for TB diagnosis that is available only in specialized, advanced laboratories (ATS, 2000). DNA amplification offers a fairly specific and sensitive diagnostic method in both pulmonary and extra-pulmonary TB, and most studies have shown it to be more sensitive than sputum smear microscopy, but less sensitive than microbial culture (Pfyffer, 1999; Magana-Arachchi et al., 2008). The specificity (ruling in disease) of the nucleic acid amplification test is high when applied to body fluids (extra-pulmonary), such as meningitis and pleural TB).

2.5.1 Limitations of the nucleic acid amplification test

The sensitivity (ruling out disease) can be compromised especially in respiratory specimens, where it can be highly variable and more inconsistent than specific (it is only about 60 percent effective under optimal conditions). This variability can be explained by the use of different cut-off values used in the different studies (Dinnes et al., 2007; Daley & Pai, 2007),

in addition to the sequence variation in both commercial and in-house assays (Whilley et al., 2008).

Evaluation of commercial nucleic acid amplification tests in both pulmonary TB and extra-pulmonary TB indicated that nucleic acid amplification tests have high, consistent specificity and positive predictive values in smear-positive patients (Ling et al., 2008; Piersimoni et al., 2002; Reischl et al., 1998; Caruyvels et al., 1996; Coll et al., 2003; Goessens et al., 2005; Miragliotta et al., 2005; Ozkutuk et al., 2006; Guerra et al., 2007; Franco-Alvarez et al., 2006); however, in smear-negative cases, when a rapid diagnostic test is needed, the accuracy of the test is more modest and variable, and the results may be influenced by patient selection and the clinical setting in which the tests are carried out (Brown et al., 1999; Barnes, 1997).

Similar results were obtained when the clinical impact of the nucleic acid amplification test systems were evaluated in low-income countries that have a high burden of TB and HIV. The nucleic acid amplification test assays used in these studies had moderate sensitivity and high specificity for TB in a predominantly HIV-seropositive population with negative sputum-smear (Davis et al., 2011; WHO, 2010).

Different laboratories report significant variability in the reproduction of this test, which can lead to false-positive results (Chedore et al., 2006); this is a major concern because of the DNA contamination of assay reagents. Even though nucleic acid amplification test techniques can amplify a small amount of genetic material, the sample must still contain a certain number of TB bacteria to be effective, and this collection is not always possible, particularly with nonpulmonary TB (Haldar et al., 2007). Therefore, it has been suggested that nucleic acid amplification tests should be combined with other diagnostic tests (for example, tests detecting INF-γ) in order to increase the sensitivity of the test (Dinnes et al., 2007). Another disadvantage of the nucleic acid amplification test is that the assay cannot distinguish dead from viable organisms, so a positive result may indicate active disease even though the TB has been cured (Manjunath et al., 1991).

Although the test itself takes little time to administer, the time required to obtain the results is considerable. Laboratories often culture the sample first, to allow the bacteria to multiply (which takes a few weeks), before carrying out the nucleic acid amplification test. The nucleic acid amplification test method requires some level of technical skill (invasive procedures are sometimes necessary to obtain samples), and is prone to cross contamination. In order to provide valid results, the nucleic acid amplification test must be run in an environment that minimizes and detects cross-contamination and test-appropriate controls. Nucleic acid amplification tests can be expensive, and in underdeveloped countries where the high-burden TB exists, commercial nucleic acid amplification tests are rarely used because of cost and complexity. Although some studies suggest that nucleic acid amplification tests are cost-effective in diagnosing TB even in low-income countries (van Cleeff et al., 2005; Dowdy et al., 2008), their use has been limited. In-house techniques that might be substituted for commercial assays often produce results that can't be validated (Daley et al., 2007). When molecular methods were compared with conventional diagnostic procedures, mostly microscopic detection, it was found that the microscopic method on its own is better than the molecular method, because of the extra care needed to interpret the results (Runa et al., 2011).

2.5.2 New approach to the nucleic acid amplification test

A more recent commercial nucleic acid amplification test (the hyplex TBC test), which meets the demand for a low-cost system, has been introduced. The hyplex test is a qualitative system for the detection of members of the Mtb complex, and it is based on a multiplex polymerase chain reaction followed by reverse hybridization to specific oligonucleotide probes and enzyme-linked immunosorbent assay (ELISA) detection. In comparison to other commercial nucleic acid amplification test systems, the hyplex TBC shows good specificity but lower sensitivity, especially with smear-negative TB specimens; it also gives false-negative results, which puts it in the same class as the other nucleic acid amplification test assays (Hofmann-Thiel et al., 2010).

These observations indicate that commercial nucleic acid amplification tests cannot replace conventional tests and cannot be used alone to confirm TB. Improvement of this technique, especially its sensitivity, is required in order for it to be beneficial for the diagnosis of TB in low-resource countries where the prevalence of disease is higher than in other parts of the world.

3. New, more specific tests

3.1 Antibody-based diagnosis assay (TB ELISA)

Recently, many new serological procedures have been evaluated for diagnosing TB. Enzyme-linked immunosorbent assays (ELISA) theoretically represent attractive serodiagnostic methods, because they are simple, rapid, inexpensive, and do not require much training or sophisticated equipment. Several researchers have tried to develop ELISA tests utilizing different antigens, such as culture filtrate, purified extracts of glycolipid, and mycobacterial sonication antigens, as well as more specific mycobacterial non-recombinant and recombinant antigens of Mtb (Daniel et al., 1986; Escamilla et al., 1996; Laal et al., 1997). Tests using such antigens were designed to detect immunoglobulins IgG, IgM, or IgA against these TB-specific antigens in whole blood, plasma, or serum of both pulmonary and extra-pulmonary TB patients (Imaz et al., 2001; Raja et al., 2002, 2004; Ramalingam et al., 2002; Zheng et al., 1994; Patil et al., 1996). Antigens from mycobacteria other than Mtb (*M. habana*) were also evaluated for their ability to diagnose extra-pulmonary TB using ELISA, and these antigens were found to be effective (Chaturvedi & Gupta 2001). To date, the 38-kDa antigen is the best candidate for the ELISA technique for diagnosing TB in actively infected individuals—but it is not reliable in extra-pulmonary or TB-HIV co-infected patients (Abebe et al., 2007). Because the available ELISA tests cannot achieve a high sensitivity, these tests are unacceptable as single diagnostic tools for TB detection (Chiang et al., 1997; Ravn et al., 2005; Weldingh et al., 2005; Araujo et al., 2004; Raja et al., 2002). The use of relatively low pure Mtb antigens has contributed to the low sensitivity of the test.

Different studies have suggested that a combination of several key antigens (antigen cocktail) may result in better sensitivity. These antigens are presumably the specific, antigens to detect the latent and early stages of the active infection in both pulmonary TB as well as extra-pulmonary TB (Houghton et al., 2002). In order to develop a successful serodiagnostic method for TB, several factors must be considered: antigen recognition by infected individuals varies depending on the stage of the disease; the heterogeneity of

human leukocyte antigen in different populations; bacterial load; and the immunological status of the patient (Abebe et al., 2007).

3.1.1 New approaches to ELISA

Recently, an ELISA test (lipoarabinomannan [LAM] antigen-detection assay) that uses urine as a sample has been developed, standardized, and is commercially available (Clearview® TB ELISA) (Boehme et al., 2005; Hamasur et al., 2001; Tessema et al., 2001). This test has many advantages: it is non-invasive, patient friendly, simple (dipstick prototype form of the test is available), rapid (requires 15 minutes to perform), easy to use, and it uses urine, which is a sterile biological fluid that is easier to obtain than sputum, which some patients have difficulties producing. Moreover, the test can be used with other body fluids, including sputum (Dheda, et al., 2010) cerebral-spinal fluid (Patel et al., 2009), and pleural fluid (Dheda K et al., 2009). However, the preliminary data indicate that the sensitivity of the urine LAM, although better than sputum microscopy in HIV-infected patients (Lawn et al., 2009), is still not adequate to replace mycobacterial culture in TB-infected patients, and the diagnostic efficacy is limited and requires further study (Gounder et al., 2011).

A promising, more rapid, and cost-effective form of ELISA has been developed: the Immunochromatographic (ICT) Test Kits; these kits detect serum antibodies against Mtb-specific antigens that are secreted by Mtb during active infection. The high sensitivity, specificity, and positive predictive values suggest that these kits are useful and simple diagnostic tools, especially for resource-poor diagnostic centers (Kumar et al., 2011).

3.2 Interferon-Gamma Release Assays (IGRAs)

Effector T cells of the cell-mediated immune response are normally present as a result of recent host encounters with antigen. T effector cells are short-lived and die off when the antigen is cleared from the host. Due to the short life of the effector T cells, their continued presence indicates that the cellular immune response is fighting a pathogen somewhere in the body. Therefore, diagnosis of an acute infection can be made by noting the presence of the antigen-specific effector T cells in a patient's blood or serum sample and by measuring the release of cytokines by the T effector cells when re-exposed to antigen in vitro. It has been shown recently that TB-specific Th1, Th22, and Th17 cells have an essential role in the immunity against TB infection; this provides a potential target for diagnosis and therapeutic intervention in TB disease (Qiao et al., 2011; Wozniak et al., 2010).

Th1 cells, which secrete INF-γ, are known to protect the body against the Mtb infection. This fact provides a unique way to examine the TB disease for diagnosis, prognosis, and treatment monitoring (Flynn et al., 1993; Boom, 1996; Gallegos et al., 2008). The production of INF-γ is influenced by many external factors, such as TB infection, and internal factors, such as interleukin (IL)-10, IL-12, IL-18, and IL-23 (Yu et al., 2011; Zhang J, et al., 2011; Han et al., 2011; Sahiratmadja et al., 2006). In fact some studies have suggested that some cytokines such as TNF-alpha, IL-2, IL-12, and IL-17 can be used to discriminate between active and latent TB disease (Sutherland et al., 2010; Schauf et al., 1993); however the exact role of each of these cytokines is not fully understood and needs to be investigated further.

Different reports have shown that the peripheral-blood mononuclear cells from patients infected with TB release INF-γ when exposed to Mtb-specific antigens *in vitro* (Ravn et al., 1999; Ulrichs et al., 1998; Lalvani et al., 2001 Mori et al., 2004). Based on these findings, different diagnostic assays have been developed to measure INF-γ released by peripheral-blood mononuclear cells cells in response to Mtb-specific antigens. Two such antigens are the early secreted antigenic target 6-kDa (ESAT-6) protein and culture filtrate protein 10-kDa (CFP-10) (Andersen et al., 2000; Tully et al., 2005). Both antigens have been shown to be important for the growth, survival, and pathogenesis of Mtb (Brodin et al., 2005; Munk et al., 2001). These proteins are secreted by Mtb in great quantities during the infection or when the bacteria are cultured *in vitro* (Andersen et al., 2000; Behr et al., 1999; Pai et al., 2004). Both ESAT-6 and CFP-10 are encoded within the region of deletion 1 (RD1) and are more specific to the organism because they are present in Mtb but are not shared with the BCG vaccine or with most of the environmental mycobacteria (Goletti et al., 2006; Sorensen et al., 1995; Harboe et al., 1996).

3.2.1 The interferon-gamma release assay tests

These discoveries have resulted in the development of two promising, blood-based, commercially available INF-γ release assay tests that have been approved for clinical use for the diagnosis of TB infection, and that use ESAT-6 and CFP-10 antigens: (1) QuantiFERON-TB Gold (QFT-G), which has been replaced in many parts of the world by a safer and simpler test method, QFT-G in-tube assay (QFT-IT) (Cellestis Limited Carnegie, Victoria, Australia) in which an additional Mtb-specific antigen TB7.7 is incorporated into the test (Syed et al., 2009; Stavri et al., 2009); and (2) T-SPOT.TB assay (Oxford Immunotech, Oxford, United Kingdom). Although the two tests share common features, they also have some technical distinctions (Richeldi, 2006). The two INF-γ release assays are designed to measure INF-γ production (INF-γ release assays) in two different ways, from peripheral-blood mononuclear cells of TB patients when exposed *in vitro* to ESAT-6 and CFP-10 proteins. QFT-G measures the quantity of INF-γ secreted by T cells, and T-SPOT.TB assay enumerates the number of TB-specific T cells secreting INF-γ after exposure to TB-specific antigens. Both of the INF-γ release assays require only a single patient visit, and the test results are available within 24 hours (Hill et al., 2004; Richeldi et al., 2004).

Many studies have shown that the QFT-G test is fairly accurate and has modest sensitivity to detect active TB (Kobashi et al., 2006; Kang et al., 2007; Pai et al., 2007). The QFT-G offers specificity of up to 97 percent in clinical trials, sensitivity of up to 89 percent, and provides clinicians with an accurate, reliable, and convenient TB diagnostic tool (Mori et al., 2004; Kobashi et al., 2006).

QFT-G has been useful for the diagnosis and differentiation between pulmonary TB and other pulmonary diseases; however it too has its limitations. Because the results depend on the clinical condition of the patients and the presence of immunosuppressive diseases, patients with localized lesions of TB infection and the elderly can sometimes get false-negative results (Kobashi et al., 2008; Kawabe, 2007).

The T-SPOT.TB test, on the other hand, quantifies the number of the INF-γ-producing TB-specific cells using a technology known as the Enzyme Linked Immunosorbent Spot (ELISPOT) assay, which is widely recognized as the most sensitive technique to measure

antigen-specific T cell function. In the T-SPOT.TB assay, recently developed by Lalvani and coworkers, individual T cells specific for the two antigens (ESAT-6 and CFP-10) are enumerated (Lalvani, Pathan et al., 2001; Lalvani, Nagvenkar et al., 2001). With this technique, peripheral-blood mononuclear cells from infected individuals are cultured overnight (16–20 hours) with ESAT-6 and CFP-10 antigens to allow the release of INF-γ by the sensitized T cells (Lalvani & Hill, 1998; Lalvani et al., 1998). A single T cell produces a dark spot, which is the footprint of an individual Mtb-specific T cell, and the number of spots is quantified.

The T-SPOT.TB technique has an estimated pooled specificity of 93 percent and up to 90 percent sensitivity for patients with culture-confirmed TB from low-incidence countries; its sensitivity, therefore, is higher than the TST (Lalvani, Pathan et al., 2001; Lalvani, Nagvenkar et al., 2001), and it has a better performance than the TST in detecting active TB (Ozekinci et al., 2007).

In addition, T-SPOT.TB detects specific T cells at frequencies as low as 1 cell per 300,000 bystander cells (Heeger et al., 2001), making the assay very sensitive for detecting immune responses even in immunosuppressed individuals actively or latently infected, in very young children, in those on anti-TNF-α treatment, in transplant and renal dialysis patients, and in pregnant women (Gebauer et al., 2002; Piana et al., 2007).

Therefore, although QFT-G has some advantages over T-SPOT.TB; for instance, it is relatively easy to perform, requires fewer steps and less-expensive equipment—which makes it more suitable for "on-filed" usage in settings with limited resources—it has been shown in different reports to be less sensitive than the T-SPOT.TB assay (Adetifa et al., 2007). It is notable that the INF-γ release assays may vary in different populations depending on various factors, including genetic background, disease epidemiology, prevalence of HIV infection, exposure to environmental mycobacteria that have similar antigens, malnutrition, and other factors (Pai et al., 2004; Dinnes et al., 2007).

3.2.2 Interferon-gamma release assays: A tool to monitor TB chemotherapy

The response of INF-γ-producing T-cells in INF-γ release assays might be related to bacterial load (Hill et al., 2005); therefore, it could be used as a quantitative surrogate marker to monitor TB chemotherapy and drug efficacy during treatment, progression, or relapses (Komiya et al., 2011; Takayanagi et al., 2011; Ribeiro et al., 2009). In addition, a strong association between the T-SPOT.TB score and the degree of sputum positivity in patients has been reported (Oni et al., 2010).

It has been suggested that INF-γ release assays can provide useful, accurate, and rapid support in the diagnosis of extra-pulmonary TB (Lai et al., 2011; Patel et al., 2010; Lai et al., 2010). Although INF-γ release assays have higher sensitivity and specificity than conventional methods, further studies are needed to evaluate their role in diagnosing children and extra-pulmonary TB infections, especially in high TB-endemic settings (Amdekar et al., 2010).

3.2.3 A comparison of the interferon-gamma release assays with the tuberculin skin test

Both INF-γ release assays (QuantiFERON and T-SPOT.TB)are beginning to replace the TST, and both assays have been approved recently for clinical use in the United States, Europe,

and Japan (FDA, 2005; Lalvani, Pathan, 2001). It has been shown that the INF-γ release assays have many advantages over the TSTs in the diagnosis of active as well as latent TB, especially in low-TB-endemic countries (Pai et al., 2004; Dinnes et al., 2007; Fukazawa, 2007; Kang et al., 2007; Ozekinic et al., 2007; Bartu et al., 2008; (Harada et al., 2008; Kabeer et al., 2010; Pia et al., 2008; Diel et al., 2009; Park et al., 2009; Toshiyama et al., 2010; Latorre et al., 2009). Since both assays are specific to Mtb and are not affected by previous exposure to environmental mycobacteria or vaccination with BCG, they have greater specificity and sensitivity than the TST in the diagnosis of latent TB in adults (Ewer et al., 2003; Shams et al., 2005; Kang et al., 2005).

In the majority of studies that compare the performance of INF-γ release assays with TSTs, INF-γ release assays seem to be significantly more accurate than TSTs and have poor agreement with it (I mean the TST), for the diagnosis of active or latent TB, in both immunocompetent or HIV-infected individuals (Rangaka et al., 2007; Mandalakas et al., 2008; Stephan et al., 2008; Jiang et al., 2009; Cağlayan et al., 2011; Cesur et al., 2010). In fact, moderate to poor diagnostic agreement between the different INF-γ release assays tests themselves has been observed (Richeldi et al., 2009; Talati et al., 2009; Latorre et al., 2010).

Nevertheless, determining the accuracy of either one of the INF-γ release assay tests to detect latent infections presents a challenge, because there is no gold standard available (Newton et al., 2008), and the only criteria that can be used is the patient's history of exposure to the disease (if known). Therefore, the accuracy and reliability of the estimated number of the global latent TB cases remains uncertain (Wiker et al., 2010). When INF-γ release assays were compared with TSTs in different longitudinal studies, INF-γ release assays may have a higher predictive value regarding the development of future TB, and unlike the TST, the INF-γ release assays (QFTGIT) results are not affected by gender or age of participants (Bakir et al., 2008; Legesse et al., 2011). Nevertheless, the decision to use INF-γ release assays instead of TSTs is often based on country guidelines and resource and logistics considerations (Cattamanchi et al., 2011).

3.2.4 The indeterminate response of interferon-gamma release assays

All INF-γ release assays are designed to include mitogen stimulation of tested cells as a positive control, along with the different TB-specific antigens used in the tests, to measure the ability of the harvested cells to produce INF-γ; when cells from tested individuals fail to respond sufficiently to either TB-specific antigens or, more specifically to the used mitogen control, the results are considered indeterminate. The indeterminate response can be explained by an error in specimen collection and handling or by the performance of the assay or T-cell anergy, which result in an inadequate response (Papay et al., 2011; Kobashi et al., 2009).

Among the different forms of the INF-γ release assays, QFT-IT has a lower rate of indeterminate results compared with T-Spot.TB, because of the simplicity of the in-tube form, which does not require as many steps as T-Spot.TB, and the fact that there is no storage of blood. T cells interact with antigens as soon as blood is collected into the QFT tubes, minimizing the potential loss of activity during storage of blood specimen.

Differences in indeterminate results have been observed among the different INF-γ release assay tests given to children; with children, both QFT-G and QFT-IT (ELISA-based assays)

are significantly more affected by indeterminate results than T-SPOT.TB (ELISPOT-based assay) (Bergamini et al., 2009). These indeterminate results can be minimized by applying the assays after acute inflammation is resolved; this later application also reduces the cost of retesting (Zrinski et al., 2011).

3.2.5 Interferon-gamma release assays and detection of TB in children

Uncertainty about the sensitivity of INF-γ release assays to detect TB in children remains an issue; the use of INF-γ release assays to detect active and latent TB infection in children, seem to perform differently. Some studies have shown that the QFT-IT has a high sensitivity and less indeterminate rates in nonimmunosuppressed children of all age groups (from 1 month to 18 years old) (Zrinski et al., 2011). However, others have shown that both forms of QuantiFERON-TB tests (QFT-G and QFT-IT) were less sensitive and can give more indeterminate results than T-SPOT.TB in children younger than 4 years old (Bergamini et al., 2009; Nicol et al., 2009; Takamatsu, 2008).

It is a priority to detect and contain the disease in this age group. Because young children have a higher chance of developing active TB than older children, as a consequence of an impaired T-cell response (Lewinsohn et al., 2004), the American Academy of Pediatrics has recently recommended that the TST continue to be used to diagnose TB in children younger than 5 years old, and that INF-γ release assays be used for children older than 5 years old (Starke, 2009; Mazurek et al., 2010). However, because of the high risk in the 5-and-under age group, it has been suggested that both tests (QFT and the TST) be used in combination, whenever it is possible. This combination of tests would improve the diagnosis of TB, and the child would be considered infected if either or both are positive (Debord et al., 2011; Pavic et al., 2011). Other studies with children older than five, HIV-infected children, or nonimmunosuppressed children, indicated that indeterminate results with QFT-G, QFT-IT, or T-SPOT.TB—were undetected or uncommon (Bergamini et al., 2009; Mandalakas et al., 2008; Tsiouris et al., 2006).

The variation in the level of cytokines (INF- γ and IL-2) released by cells after stimulation with QFT antigens in children, is age dependent; it can identify those children with latent TB who are younger than 5 years old from those older than 5 years old (Lighter-Fisher et al., 2010).

3.2.6 Interferon-gamma release assays for testing TB in children with cancer

Both the TST and the INF-γ release assays were used to detect TB in children with cancer before their initial chemotherapy. All tests performed suboptimally, and therefore none of them can be used individually to confirm or disprove TB infection (Stefan et al., 2010).

3.2.7 Interferon-gamma release assays and screening for latent TB

In adult patients, latent TB can be detected more effectively with INF-γ release assays than with the TST; QFT-G and QFT-IT can be used for diagnosis and T-SPOT.TB for exclusion (Chang & Leung 2010). However, in some settings, both tests are used to screen for TB (Torres Costa et al., 2011; Katsenos et al., 2011).

Although INF-γ release assays are affected by cellular immune statutes and age, they demonstrate low agreement with the TST and perform better in detecting latent TB in adult patients (Santín Cerezales 2011; Zhao et al., 2011). Both of the INF-γ release assays (T-SPOT.TB and QFT) require only a single patient visit, and the test results are available within 24 hours (Hill et al., 2004; Richeldi et al., 2004).

The Centers for Disease Control and Prevention, USA, recommend that INF-γ release assays can replace the TST (single screening strategy) in all settings (Mazurek et al., 2005). However, recent UK TB guidelines advise screening for latent TB using the TST, followed by INF-γ release assays if the TST is positive (dual screening) (Leyten et al., 2007; Sauzullo et al., 2011; Dosanjh et al., 2008;148: Ritz et al., 2011). The dual screening strategy has been reported to be more cost-effective than the single screening strategy (INF-γ release assay or TST alone) for screening latent TB; however this conclusion and the interpretation of results is relative to the prevalence of TB in the setting as well as the length of contact with the infection (Pooran et al., 2010).

3.2.8 Drawbacks of interferon-gamma release assays

One limitation of the INF-γ release assays is that they are inconsistent in detecting TB in HIV patients. Some reports indicate that they are not the best tools for diagnosing TB among HIV-infected individuals with advanced immunodeficiency diseases, because of low sensitivity resulting from the low T-cell count (Chen et al., 2011). However, other studies reported that the sensitivity of the T-SPOT.TB assay in detecting TB in active HIV disease may be not highly impaired by advanced immunosuppression (Oni et al., 2010).

Another limitation of INF-γ release assays is their inability to distinguish active from past-treated TB infections (Kim et al., 2011; (Kim et al., 2010). The role of INF-γ release assays to distinguish between latent TB and active TB and their predictive ability of the progression of latent TB to active TB infection needs to be studied further, especially in high-burden settings (Dheda et al., 2009). Therefore, although INF-γ release assays have been approved in many countries to diagnose latent TB, especially in adults, this test still has little clinical value in the diagnosis of active TB (Dominguez et al., 2009). Nevertheless, growing evidence supports the idea that recruiting Mtb-specific T cells in active TB from fluids at the "local" sites of infection, such as pleural effusion (Wilkinson et al., 2005; Barnes et al., 1993; Barnes et al., 1989), cerebrospinal fluid (Thomas et al., 2008), ascites (Wilkinson et al., 2005), pericardial fluid (Biglino et al., 2008), and bronchoalveolar lavage (BAL) (Jafari et al., 2006; Jafari et al., 2009) is more effective than blood, in the diagnosis of extra-pulmonary TB. In this way the INF-γ release assays are looking at the "local " site of infection rather than "systemic" Mtb-specific immune response in blood, which may only provide background information about effector memory T-cells in active TB. This provides a promising approach to distinguish active TB from latent TB in routine clinical practice (Jafari et al., 2009).

The cut-off values currently recommended by the manufacturers are being disputed in some studies (Soysal et al., 2008). Consideration of new (low) cut-off values for both T-SPOT.TB and QFT, which may improve the assays' sensitivity, are now recommended, especially in intermediate- and high-endemic areas of TB and HIV (Soysal et al., 2008; Kanunfre et al., 2008; Legesse et al., 2010). The relative complexity of the INF-γ release assays can result in technical errors at many levels, resulting from insufficient cells,

reduced cells activated due to prolonged transport or storage of blood, improper handling of specimens, the presence of INF-γ antibodies, and the incorrect addition of mutagen. Any of these technical problems can contribute to the invalidity and inaccuracy (unusual INF-γ measurements) of the test results, adding to its potential disadvantages (Kampmann et al., 2005; Powell et al., 2011).

The level of complexity mentioned above in addition to the requirement for special equipment, skilled laboratory personnel, and the high cost of the INF-γ release assays are among the limitations of the assays, which should be strongly considered, especially in low-resources settings. INF-γ release assays are recommended for use as a confirmation tool when a patient with negative TST is suspected of having TB, or to exclude a positive TST result from BCG vaccination (Sun et al., 2010).

3.2.9 Interferon-gamma release assays and detection of TB in patients with immune-mediated inflammatory diseases

Studies involving immune-mediated inflammatory diseases (such as psoriasis, rheumatoid arthritis, etc.) indicated that INF-γ release assays are superior to the TST for detecting latent TB in both endemic and non-endemic areas (Ponce de Leon et al., 2005; Ponce de Leon et al., 2008; Sellam et al., 2007; Murakami et al., 2009; de Andrade et al., 2011).

Since most of the information on the performance of INF-γ release assays have been gathered from studies done in developed countries, it is important that further research on adults and children be done in developing countries where TB is endemic. These studies should include the presence of additional factors that have been reported to cause false negative results as high as 35.5 percent; these factors include HIV infection, malnutrition, impaired immune status, age, and the different ethnic backgrounds of patients (Pai et al., 2006; Menzies et al., 2007; Im et al., 1991; Landis & Koch, 1977; Legesse et al., 2010; Legesse et al., 2011 Apr 9;11:89).

Despite the progress that has been made in studying the use of INF-γ release assays, additional research is still required to study further the limitations of the assays and the ways to overcome them to improve the best utility of the tests in diagnosing and controlling TB (Lalvani & Pareek 2010; Mazurek et al., 2010).

4. Conclusions

Despite the continued research to identify the key Mtb antigens and biomarkers for developing the ideal diagnostic test, there is not yet a rapid, reliable, and economical method to diagnose both active and latent TB. The importance of diagnosing latent TB is often overshadowed in many parts of the world; however, it is key to controlling the spread of infection. In the last few years, new tests have been developed based on significant advances in understanding the genomic and immunology of Mtb. The new tests include the nucleic acid amplification test, QuantiFERON-TB and T-SPOT.TB, which have the advantages of higher specificity and sensitivity than the conventional tests—important for physicians so that they can avoid the inappropriate treatment of false-positive vaccinated individuals. Yet these new tests have disadvantages as well, including cost, training, and complexity. None of the available microbiological or immunological tests alone can

accurately confirm the TB infection, while waiting for the culture results which can take weeks. Given the global burden of this disease, and its potential to spread rapidly, the importance of developing a novel assay or improving the existing methods for TB detection, active and latent, has never been greater.

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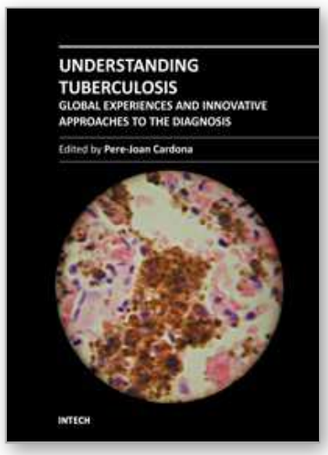

Understanding Tuberculosis - Global Experiences and Innovative Approaches to the Diagnosis

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Mycobacterium tuberculosis is a disease that is transmitted through aerosol. This is the reason why it is estimated that a third of humankind is already infected by Mycobacterium tuberculosis. The vast majority of the infected do not know about their status. Mycobacterium tuberculosis is a silent pathogen, causing no symptomatology at all during the infection. In addition, infected people cannot cause further infections. Unfortunately, an estimated 10 per cent of the infected population has the probability to develop the disease, making it very difficult to eradicate. Once in this stage, the bacilli can be transmitted to other persons and the development of clinical symptoms is very progressive. Therefore the diagnosis, especially the discrimination between infection and disease, is a real challenge. In this book, we present the experience of worldwide specialists on the diagnosis, along with its lights and shadows.

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